Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides methods for detecting epidermal growth factor (EGF) RNA, epidermal growth factor receptor (EGFr) RNA, her-2/neu RNA, c-myc RNA, or heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1) RNA, or any combination thereof in bodily fluids in an animal, most preferably a human. These methods are useful, *inter alia*, for detecting cancerous or precancerous cells in the animal.

In preferred embodiments of the methods of the invention, mammalian RNA in a bodily fluid, a portion thereof comprising extracellular EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA hnRNP A2/B1 RNA, or any combination thereof is extracted from said bodily fluid. This extracted RNA is then amplified, either after conversion into cDNA or directly, using *in vitro* amplification methods in either a qualitative or quantitative manner, and using oligonucleotide primers specific for EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA or hnRNP A2/B1 RNA or any combination thereof, or cDNA derived therefrom, to form a product DNA fragment having a size and sequence complexity specific for each of said specific RNAs. The amplified product is then detected in either a qualitative or a quantitative manner.

In the practice of the methods of the invention, mammalian RNA, a portion of which comprises extracellular EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, hnRNP A2/B1 RNA, or any combination thereof, is extracted from a bodily fluid, including but not limited to whole blood, plasma, serum, urine, effusions, ascitic fluid, saliva, cerebrospinal fluid, cervical secretions, vaginal secretions, endometrial secretions, amniotic fluid, gastrointestinal secretions,

bronchial secretions including sputum, breast fluid or secretions or washings. Extraction can be performed using, *for example,* extraction methods described in co-owned and co-pending U.S. Patent Application Serial No. 09/155,152, the entire disclosure of which is hereby incorporated by reference and include but are not limited to gelatin extraction methods; silica, glass bead, or diatom extraction methods; guanidinium thiocyanate acid-phenol based extraction methods; guanidinium thiocyanate acid based extraction methods; methods using centrifugation through cesium chloride or similar gradients; phenol-chloroform based extraction methods; or other commercially available RNA extraction methods. Alternatively, extraction may be performed using probes that specifically hybridize to a particular RNA, more preferably using isolation methods dependent thereupon, *for example* chromatographic methods and methods for capturing RNA hybridized to said specific primers. In a preferred embodiment, the bodily fluid is either blood plasma or serum. It is preferred, but not required, that blood be processed soon after drawing, and preferably within three hours, to minimize any degradation in the sample. In a preferred embodiment, blood is first collected by venipuncture and kept on ice until use. Preferably within 30 minutes of drawing the blood, serum is separated by centrifugation, for example at 1100 x *g* for 10 minutes at 4 degrees centigrade. When using plasma, blood should not be permitted to coagulate prior to separation of the cellular and acellular blood components. Serum or plasma can be frozen, for example at –70 degrees centigrade after separation from the cellular portion of blood, until use. When using frozen blood plasma or serum, the frozen plasma or serum is rapidly thawed, for example in a water bath at 37 degrees centigrade, and RNA is extracted therefrom without undue delay, most preferably using a commercially available kit (for example the Perfect RNA Total RNA Isolation Kit obtained from Five Prime – Three Prime, Inc., Boulder, Colorado), according to the manufacturer's instructions. Other

alternative and equivalent methods of RNA extraction are further provided in co-owned and co-pending U.S. Patent Application Serial No. 09/155,152, incorporated herein by reference in its entirety.

Following extraction of RNA from a bodily fluid that contains EGF mRNA, EGFr mRNA, her-2/neu mRNA, c-myc mRNA, or hnRNP A2/B1 RNA, or any combination thereof, the EGF RNA, EGFr RNA, her-2/neu RNA, c-myc RNA, or hnRNP A2/B1 RNA or cDNA derived therefrom is amplified *in vitro*. Applicable amplification assays are detailed in co-owned and co-pending U.S. Patent Application Serial No. 09/155,152, as herein incorporated by reference, and include but are not limited to reverse transcriptase polymerase chain reaction (RT-PCR), ligase chain reaction, DNA signal amplification, amplifiable RNA reporters, Q-beta replication, transcription-based amplification, boomerang DNA amplification, strand displacement activation, cycling probe technology, isothermal nucleic acid sequence based amplification, and other self-sustained sequence replication assays.

In preferred embodiments of the methods of the invention, RNA encoding EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1, or any combination thereof is converted into cDNA using reverse transcriptase prior to *in vitro* amplification using methods known in the art. For example, a sample such as 10 microL extracted serum RNA is reverse-transcribed in a 30 microL volume containing 200 Units of Moloney murine leukemia virus (MMLV) reverse transcriptase (Promega, Madison, WI), a reaction buffer supplied by the manufacturer, 1 mM each dNTPs, 0.5 micrograms random hexamer oligonucleotide primers, and 25 Units of RNAsin (Promega, Madison, WI). Reverse transcription is typically performed under an overlaid mineral oil layer to inhibit evaporation, and incubated at room temperature for 10 minutes followed by incubation at 37 degrees C for one hour. In another embodiment, reverse transcription is performed by the